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## Co-occurrence of aminoglycoside and $\beta$ -lactam resistance mechanisms in aminoglycoside- non-susceptible *Escherichia coli* isolated in the Zurich area, Switzerland

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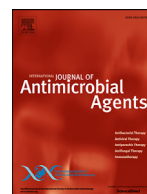


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# Co-occurrence of aminoglycoside and $\beta$ -lactam resistance mechanisms in aminoglycoside- non-susceptible *Escherichia coli* isolated in the Zurich area, Switzerland

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## ABSTRACT

The co-occurrence of aminoglycoside and  $\beta$ -lactam resistance was assessed in 3358 consecutive *Escherichia coli* clinical isolates collected in 2014 in the greater Zurich area, Switzerland. Non-susceptibility to at least one of the tested aminoglycosides was observed in 470/3358 *E. coli* strains (14%). In strains categorized as broad-spectrum  $\beta$ -lactamase (BSBL)-producers (1241/3358 isolates), extended-spectrum  $\beta$ -lactamase (ESBL)-producers (262/3358) and AmpC-producers (66/3358), resistance to aminoglycoside was found in 23%, 52% and 20% of the isolates, respectively. In contrast, aminoglycoside-susceptible strains were rarely resistant to  $\beta$ -lactams (33/1777, 1.9%). The genomes of 439 aminoglycoside-resistant *E. coli* were sequenced and aminoglycoside and  $\beta$ -lactam genotypes were analysed. The most prevalent aminoglycoside resistance genes were *aph(3')-Ia* (133 strains, 30.3%), *aac(3)-IId* (100 strains, 22.8%), and *aac(6')-Ib-cr* (52 strains, 11.8%). The most frequent associations with  $\beta$ -lactam resistance genes were *aph(3')-Ia* or *aac(3)-IId* with *bla<sub>TEM-1</sub>* (94 and 72 strains, respectively), and *aac(3)-IId/aac(6')-Ib-cr* with *bla<sub>CTX-M-15</sub>/bla<sub>OXA-1</sub>* (23 strains). These results indicate a frequent association of *aac(3)* and *aph(3')* genotypes with BSBL production, and a frequent co-occurrence of *aac(6')* genes with ESBL production. The high rate of co-occurrence of aminoglycoside resistance and  $\beta$ -lactamase production must be considered in combination therapy.

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## 1. Introduction

Aminoglycosides are an important class of bactericidal antibiotics that are frequently used, mostly in combination with  $\beta$ -lactams, to treat severe infections caused by Gram-negative bacteria [1]. Resistance to aminoglycosides has been increasingly reported, including, most worryingly, in association with that to other antibiotic classes, such as  $\beta$ -lactams and fluoroquinolones [2–5].

Resistance to aminoglycosides in Gram-negative bacteria is mainly due to the production of aminoglycoside-modifying enzymes (AMEs) [1,6] or modification of the ribosome by acquired 16S rRNA methyltransferases (RMTases) [6,7]. AME production is by far the most frequent resistance mechanism in *E. coli*. AMEs

are divided into three classes according to the reaction they catalyse: (i) aminoglycoside *N*-acetyltransferases (AAC), (ii) aminoglycoside *O*-phosphotransferases (APH) and (iii) aminoglycoside *O*-nucleotidyltransferases (ANT) [1]. AMEs can modify aminoglycosides at various sites of the drug scaffold and the enzymes are classified into subclasses and types according to different substrate profiles. For example, AAC(3) acetylates the amino group at position 3 of the central 4,6-di-substituted deoxystreptamine ring II, AAC(6') acetylates the amino group at position 6' of ring I and APH(3') phosphorylates the hydroxyl group at position 3' of ring I of the aminoglycoside. AMEs frequently modify more than one aminoglycoside and the same aminoglycoside can be affected by several enzymes. Lastly, aminoglycoside modification may not always result in recognizable phenotypic resistance as determined in vitro by assessment of minimal inhibitory concentrations (MICs) [1,8].

Hydrolysis of  $\beta$ -lactams by  $\beta$ -lactamases is the most common resistance mechanism to this drug class in Enterobacterales [6,9].  $\beta$ -lactamases can be classified based on molecular char-

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acteristics or functional properties. Molecular characterization is based on amino acid sequence and conserved motifs and divides  $\beta$ -lactamases into four classes (A, B, C and D) [6,10]. The functional characterization scheme, used in this work, classifies  $\beta$ -lactamases based on their substrate and inhibitor profiles [6,11]. Major functional groups include broad-spectrum  $\beta$ -lactamases (BSBL, i.e., hydrolysing penicillins and first- and second-generation cephalosporins), extended spectrum  $\beta$ -lactamases (ESBL, i.e., additionally hydrolysing third-generation cephalosporins and monobactams and inhibited by  $\beta$ -lactamase inhibitors), AmpC cephalosporinases (i.e., hydrolysing penicillins and cephalosporins), and carbapenemases (i.e., hydrolysing virtually all  $\beta$ -lactams) [6].

The structural genes for AMEs and  $\beta$ -lactamases are often part of mobile genetic elements carried by a variety of plasmids in combination with resistance genes to other drug classes, resulting in multidrug-resistant isolates [9]. Particularly worrisome is the frequent co-occurrence of RMTases and metallo- $\beta$ -lactamases [7]. The ever-growing problem of multidrug resistance and the need for carbapenem-sparing regimens to treat infections caused by ESBL-producing Enterobacteriaceae have revived interest in aminoglycosides and efforts to detect and identify the resistance mechanisms against this drug class [8,12]. Although there is abundant literature on the microbiological, clinical and epidemiological aspects of  $\beta$ -lactam resistance in Enterobacteriaceae, there is little information about aminoglycoside resistance.

The aim of this study was to assess the aminoglycoside and  $\beta$ -lactam resistance rates in clinical *E. coli* isolated in the Zurich metropolitan area in 2014, and to investigate the co-occurrence of aminoglycoside and  $\beta$ -lactam resistance genes.

## 2. Materials and Methods

### 2.1. Clinical isolates

All 5765 *E. coli* collected during 2014 from various clinical materials in the diagnostic laboratory of the Institute of Medical Microbiology, University of Zurich were included in the study (Figure S1). When more than one *E. coli* was isolated from the same patient, only the first strain was included. If aminoglycoside-susceptible and -resistant strains were recovered from the same patient, only the first aminoglycoside-resistant isolate was studied. Each patient was included in the analysis only once. Of the resulting 3358 non-duplicate *E. coli*, 470 had growth inhibition diameters below the cut-off of at least one of the tested aminoglycosides, gentamicin, tobramycin and kanamycin. A total of 461 aminoglycoside non-wild-type strains were sequenced (Figure S1) and screened for the presence of aminoglycoside or  $\beta$ -lactam resistance genes.

### 2.2. MALDI-TOF mass spectrometry identification

MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) identification was performed using the direct formic acid transfer method [13].

### 2.3. Antibiotic susceptibility testing (AST)

AST was performed by disk diffusion according to EUCAST guidelines [14]. Aminoglycoside susceptibility profiles were evaluated by disk diffusion according to EUCAST epidemiological cut-offs (ECOFFs, gentamicin and tobramycin = 16 mm [15]) or local ECOFF (kanamycin = 15 mm) [16]. A cefpodoxime cut-off of 21 mm was used for screening of ESBL production. Carbapenemase production was suspected if the meropenem inhibition zone was below 25 mm, or if the meropenem inhibition zone was between

25 and 28 mm and the piperacillin/tazobactam inhibition zone diameter was below 17 mm according to EUCAST [17]. Screening for AmpC, ESBL and carbapenemase production was performed according to phenotype-based algorithms described previously [18–20]. In brief, AmpC production was suspected if cefoxitin inhibition zones were below 19 mm. Results were confirmed using combination disk testing: for AmpC the difference between cefoxitin with and without cloxacillin was measured; for ESBL the difference was determined between cefotaxime/clavulanic acid vs. cefotaxime, and ceftazidime/clavulanic acid vs. ceftazidime. Strains resistant to any  $\beta$ -lactam, but not producing an AmpC, ESBL or carbapenemase, were classified as BSBL. During 2014, all 3358 *E. coli* were tested for gentamicin and tobramycin susceptibility. In addition, 3011 strains were tested for kanamycin susceptibility.

### 2.4. Whole-genome sequencing (WGS)

DNA libraries were prepared following the Illumina Nextera protocol (Illumina, San Diego, CA, USA) or the QIAseq FX DNA Library Kit (QIAGEN AG, Hombrechtikon, Switzerland). Quality control of the library was performed using capillary electrophoresis (Fragment Analyzer Automated CE System by Advanced Analytical). Sequencing was done on either the HiSeq 1500 or MiSeq platform (Illumina).

### 2.5. Detection of resistance genes

The fastq sequence files were processed by the ARIBA pipeline [21] and Resistance Gene Identifier 4.0.1 [22]. Resistance genes were identified using ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation) [23] and CARD (Comprehensive Antibiotic Resistance Database) [22].

### 2.6. Sequence type (ST) analysis

Sequence typing was performed according to the Warwick scheme [24] with RidomSeqSphere software 4.1.9 (Ridom GmbH, Muenster, Germany).

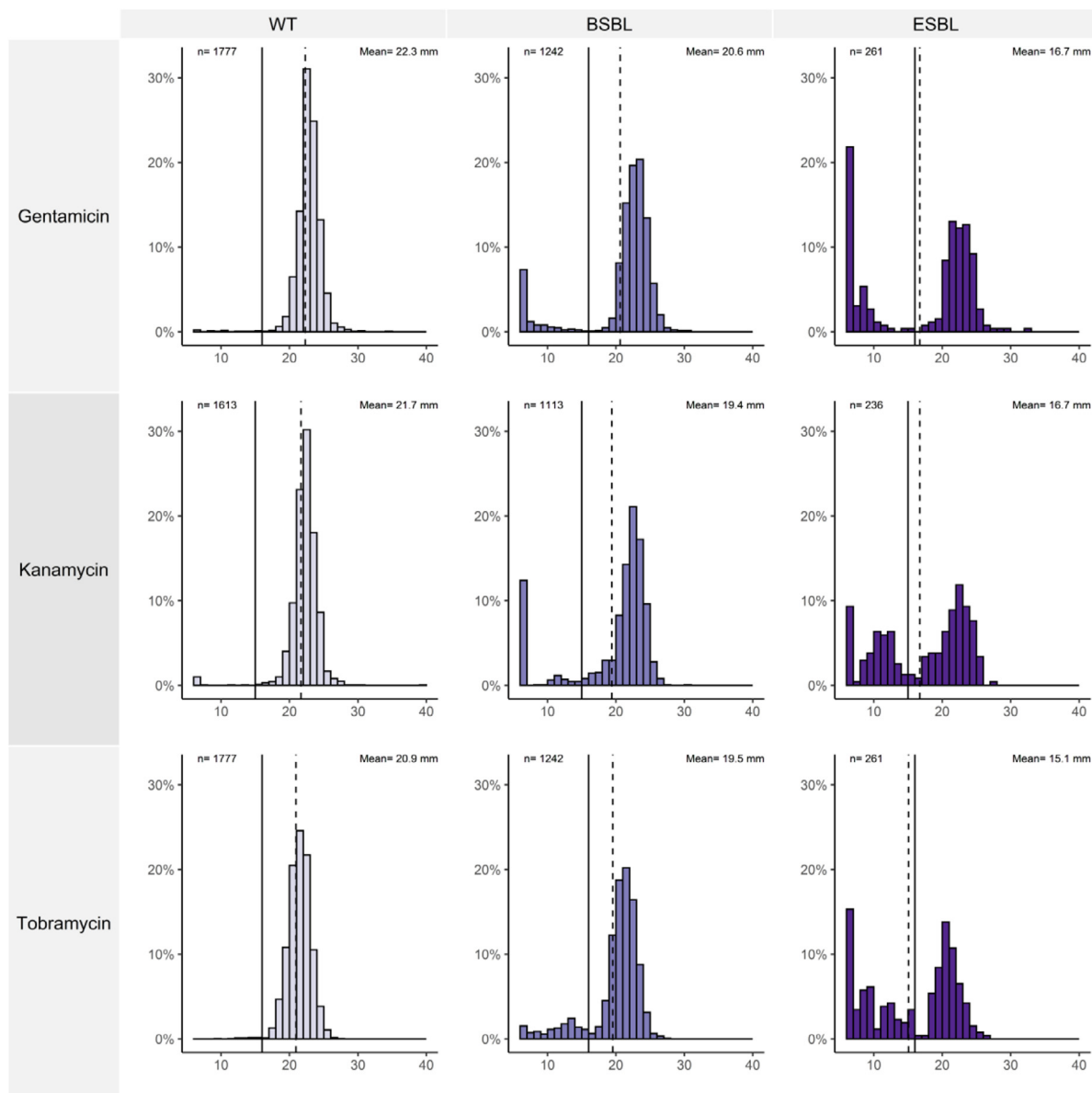
### 2.7. Statistical analysis

R (version 3.6.1) was used for statistical analysis [25]. Fisher's exact test, R base version, was used. A *P*-value below 0.05 was considered statistically significant.

## 3. Results and Discussion

### 3.1. Phenotypic aminoglycoside resistance

ECOFFs were used to screen for aminoglycoside-non-susceptible *E. coli* as ECOFFs separate wild-type from non-wild-type populations more accurately than clinical breakpoints (CBPs) [6,8,26]. A total of 5765 *E. coli* strains collected from various clinical materials during 2014 were analysed. When more than one *E. coli* strain was isolated from the same patient, only the first aminoglycoside-resistant strain, if available, was included. Of 3358 clinical isolates, 2888 (86%) were susceptible to gentamicin, tobramycin and kanamycin. The remaining 470 strains (14%) were resistant to at least one aminoglycoside (Fig. 1 and Table 1). Seven aminoglycoside resistance phenotypes were observed (Table S1). The resistance rate observed is somewhat higher than that reported in 2015 by ANRESIS (8.9%), the Swiss centre for antibiotic resistance for Switzerland [27], and this may be due to several reasons. First, the ANRESIS report includes strains isolated from hospitalized patients and outpatients, whereas most of the *E. coli* analysed in the current study were collected from the University Hospital of Zurich, a



**Fig. 1.** Gentamicin, tobramycin and kanamycin inhibition zone diameters [mm] of 3358 unique *E. coli* grouped by  $\beta$ -lactam resistance mechanism. BSBL, broad-spectrum  $\beta$ -lactamase; ESBL, extended-spectrum  $\beta$ -lactamase; WT, wild-type. AmpC, AmpC/ESBL co-producers and carbapenemase-producers were not included in the figure. The dashed line indicates the arithmetic mean of each distribution. The solid black line indicates the ECOFF: gentamicin and tobramycin 16 mm, kanamycin 15 mm. The total number (n) and the mean of each group are given at the top.

**Table 1**

Aminoglycoside non-susceptibility rates grouped by  $\beta$ -lactam resistance mechanism in 3358 unique *E. coli*.

$\beta$ -lactam resistance phenotype (Total)	Wild-type (1777)	BSBL (1241)	ESBL (262)	AmpC (66)	AmpC/ ESBL (10)	CPE (2)	Total (3358)
Gentamicin non-susceptible strains	15 (0.8%)	149 (12%)	95 (36.3%)	9 (13.6%)	1/10	1/2	270 (8%)
Kanamycin non-susceptible strains	19 (1.1%)	177 (14.3%)	93 (35.5%)	7 (10.6%)	4/10	1/2	301 (9%)
Tobramycin non-susceptible strains	12 (0.7%)	158 (12.7%)	125 (47.7%)	11 (16.7%)	4/10	1/2	311 (9.3%)
Aminoglycoside non-susceptible strains	33 (1.9%)	282 (22.7%)	137 (52.3%)	13 (19.7%)	4/10	1/2	470 (14%)

BSBL, broad-spectrum  $\beta$ -lactamase; CPE, carbapenemase-producing *Enterobacteriaceae*; ESBL, extended-spectrum  $\beta$ -lactamase.

tertiary care hospital. Second, there is considerable variation in the prevalence of aminoglycoside-resistant *E. coli* strains among Swiss regions [28].

Non-susceptibility to gentamicin, tobramycin or kanamycin individually was found in 270 (8%), 311 (9.3%) and 301 (9.0%) strains, respectively. These rates are comparable to those reported by the ECDC for Europe in 2014; e.g., France, Germany and Austria have resistance rates to tobramycin and/or gentamicin of 7.7%, 6.9% and 6.9% in invasive *E. coli* isolates, respectively [29].

### 3.2. Phenotypic resistance to $\beta$ -lactams

The rate of phenotypic  $\beta$ -lactam resistance was examined using phenotype-based algorithms [18–20]. Thus, 1777/3358 *E. coli* isolates (52.9%) were categorized as  $\beta$ -lactam wild-type (i.e., susceptible to all  $\beta$ -lactams), 1241 (37%) as BSBL-producers, 262 (7.4%) as ESBL-producers, and 66 (2%) as AmpC-producers (Table 1). In addition, 10 strains producing ESBL/AmpC and two producing carbapenemases (CPE) were detected. Reported ESBL rates in central

Europe for *E. coli* blood stream isolates (BSI) range from 7.5% to 10.5% [30,31]. Although the isolates in the current study were not restricted to BSI, the rate of ESBL-producing strains was comparable.

### 3.3. Phenotypic co-resistance to aminoglycosides and $\beta$ -lactams

Determining the phenotypic co-occurrence of aminoglycoside and  $\beta$ -lactam resistances showed only 33 of 1777  $\beta$ -lactam wild-type strains (1.9%) were aminoglycoside-resistant (Table 1 and Fig. 1). In contrast, 282 of 1241 (22.8%) BSBL-producers were resistant to one or more aminoglycosides, and more than half of all ESBL-producers, i.e., 137 of 262 (52.3%), were aminoglycoside-resistant. Of the 66 AmpC-producers, 13 (19.7%) were aminoglycoside-resistant. Resistance to tobramycin, kanamycin and gentamicin was observed in 47.7%, 39.2% and 36.3% of ESBL-producers, respectively. Marginally different rates were found in a multicentre study conducted in Spanish hospitals in 2006: the percentage of *E. coli* ESBL-producers non-susceptible to tobramycin, kanamycin and gentamicin was 31.3%, 28.2% and 25.8%, respectively [3]. Thus, on average, aminoglycoside resistance was 10–20 times more prevalent in strains resistant to  $\beta$ -lactams compared with  $\beta$ -lactam-susceptible strains.

### 3.4. Identification of aminoglycoside resistance genes

The aminoglycoside resistance genes present in the current study isolates were then investigated. Altogether, the resistance mechanisms in 439 *E. coli* clinical isolates were determined by WGS and are given in Table 2. Unfortunately, nine strains were no longer available and a further 22 strains were excluded for phenotype-genotype discrepancies. Overall, 31 resistance genotypes were found. Fourteen consisted of a single determinant specifying an AME and 17 consisted of various gene combinations of AMEs and 16S rRNA methyltransferases. The most prevalent genes for individual AMEs were *aph(3')-Ia* (133/439, 30.3%), *aac(3)-II* (100/439, 22.8%) and *aac(6')-Ib-cr* (52/439, 11.8%) (Table S2).

In a study conducted by Miró et al., 264 aminoglycoside non-susceptible *E. coli* clinical isolates collected in a Spanish hospital in 2006 were analysed and the most prevalent AME genes conferring resistance to gentamicin, tobramycin and amikacin were *aph(3')-Ia* (13.9%), *aac(3)-IIa* (12.4%), and *aac(6')-Ib* (4.2%) [32]. Interestingly, despite the different criteria used to select the strains (ECOFF vs. CBP), the relative prevalence of the resistance mechanisms was similar to that in the current study. In another work in 2009, the most frequent AMEs in a collection of 105 *E. coli* resistant or intermediately resistant to gentamicin and/or tobramycin were AAC(3)-II (66.7%) and AAC(6')-Ib (10.2%) [33]. Of note, in the latter work neither the susceptibility to kanamycin nor the presence of the *aph(3')-I* gene were investigated.

### 3.5. Co-occurrence of aminoglycoside and $\beta$ -lactam resistance genes

The presence of  $\beta$ -lactam resistance genes was investigated in the 439 aminoglycoside non-susceptible *E. coli* (Table 2). Due to their small numbers, 11 AmpC and 4 ESBL/AmpC-producers were not included in Fig. 2 and will not be discussed further. Fig. 2 shows the co-occurrence of aminoglycoside and  $\beta$ -lactam resistance genes in 424 strains classified as  $\beta$ -lactam wild-type, or BSBL- or ESBL-producers (Fisher's exact test  $P=0.0004998$ ). Based on genotypic data, the isolates were grouped by aminoglycoside resistance mechanism conferring the same inferred resistance phenotype (Table 3). Thus, all strains only carrying an *aph(3')* gene were classified as kanamycin-resistant [34]. Strains harbouring an *aac(3')* gene were considered as resistant to gentamicin, kanamycin and tobramycin [8]. Resistance to kanamycin,

tobramycin and amikacin was inferred from the presence of an *aac(6')* gene [6,8].

Most isolates producing APH(3')-Ia or AAC(3)-II alone were BSBL-producers; 107/132 (81.1%) and 96/135 (71.1%) respectively, and mostly associated with TEM-1;  $n=97$  and 94, respectively. In strains with AME combinations without AAC(6') or RMTases, the large majority were BSBL-producers (29/34, 85.3%). These combinations were predominantly composed of *aac(3)* and *aph(3')* genes. Again, the most prevalent  $\beta$ -lactam resistance mechanism was TEM-1 ( $n=28$ ).

In contrast, most strains harbouring an *aac(6')-Ib* alone were ESBL-producers; 32/50 (64%). These were mostly CTX-M-15/OXA-1 co-producers ( $n=19$ ). A further 18/50 (36%) strains produced a BSBL, of these all but one produced OXA-1. No strain was  $\beta$ -lactam wild-type. Within the 73 isolates with AME combinations including AAC(6') or RMTases, the largest proportion were ESBL-producers (57/73, 78.1%), with CTX-M-15/OXA-1 prevailing ( $n=31$ ). Another 16 of 73 (21.9%) strains produced BSBL, these were mostly co-expressing TEM-1 and OXA-1. Again, no strain was  $\beta$ -lactam wild-type.

The common association of AAC(6')-Ib-cr enzymes with CTX-M/OXA-1 was intriguing, prompting more detailed study of this correlation (Fig. 3 and Table S4). Of 121 AAC(6')-Ib-cr-producing strains only 9 did not contain an OXA-1. Dismissing TEM-1 and AAC(3)-II, the most frequent combinations were AAC(6') and  $\beta$ -lactamases CTX-M-15/OXA-1/AAC(6')-Ib-cr (62/121, 51.2%), OXA-1/AAC(6')-Ib-cr 26 (23.1%) and CTX-M-1/OXA-1/AAC(6')-Ib-cr 18 (14.9%) (Table S4). This frequent trio, CTX-M-15/OXA-1/AAC(6')-Ib-cr, confers broad-spectrum resistance towards aminoglycosides,  $\beta$ -lactams and fluoroquinolones [35,36].

In a previous study, 105 of 257 *E. coli* isolates resistant to amoxicillin/clavulanic acid were also resistant to at least one aminoglycoside [2]. Of 15 strains producing TEM-1 alone, eight carried *aph(3')-Ia*, four carried *aac(3)-IIa* and one isolate harboured *ant(2'')-Ia*. Among 30 OXA-1 producing strains, four harboured an *aac(6')-Ib* gene and three a combination of *aac(6')-Ib* and *aph(3')-Ia*. Contrary to the current findings, in 21/30 strains the OXA-1 enzyme was associated with an ANT(2'')-Ia. Curiously, in strains carrying a combination of ESBL and OXA-1, co-occurrence with AAC(6')-Ib was found in 23/24 strains. Although these observations point to an association of  $\beta$ -lactam and aminoglycoside resistance mechanisms, the full extent of this association can only be assessed by studying large numbers of corresponding isolates by WGS, as in the current study.

### 3.6. Co-localization of aminoglycoside and $\beta$ -lactam resistance genes

To address whether the described resistance mechanisms are located on a common mobile element, long-read sequencing is necessary. Although we do not have this data, the carriage of *aac(6')-Ib-cr*, *bla*<sub>CTX-M-1/9/15</sub>, *bla*<sub>TEM-1</sub>, and *bla*<sub>OXA-1</sub> on IncF plasmids has been well established, particularly in association with FII, FIA and FIB replicons [37]. Incorporation of *aac(6')-Ib-cr/bla*<sub>CTX-M-15/bla<sub>OXA-1</sub> in an IncFII plasmid is common [36]. This probably explains the high co-occurrence of CTX-M-15/OXA-1/AAC(6')-Ib-cr observed in the current study. Similar multidrug-resistance plasmids, encoding combinations of TEM-1 with AAC(3) and/or APH(3'), may be responsible for most co-occurrences in this study.</sub>

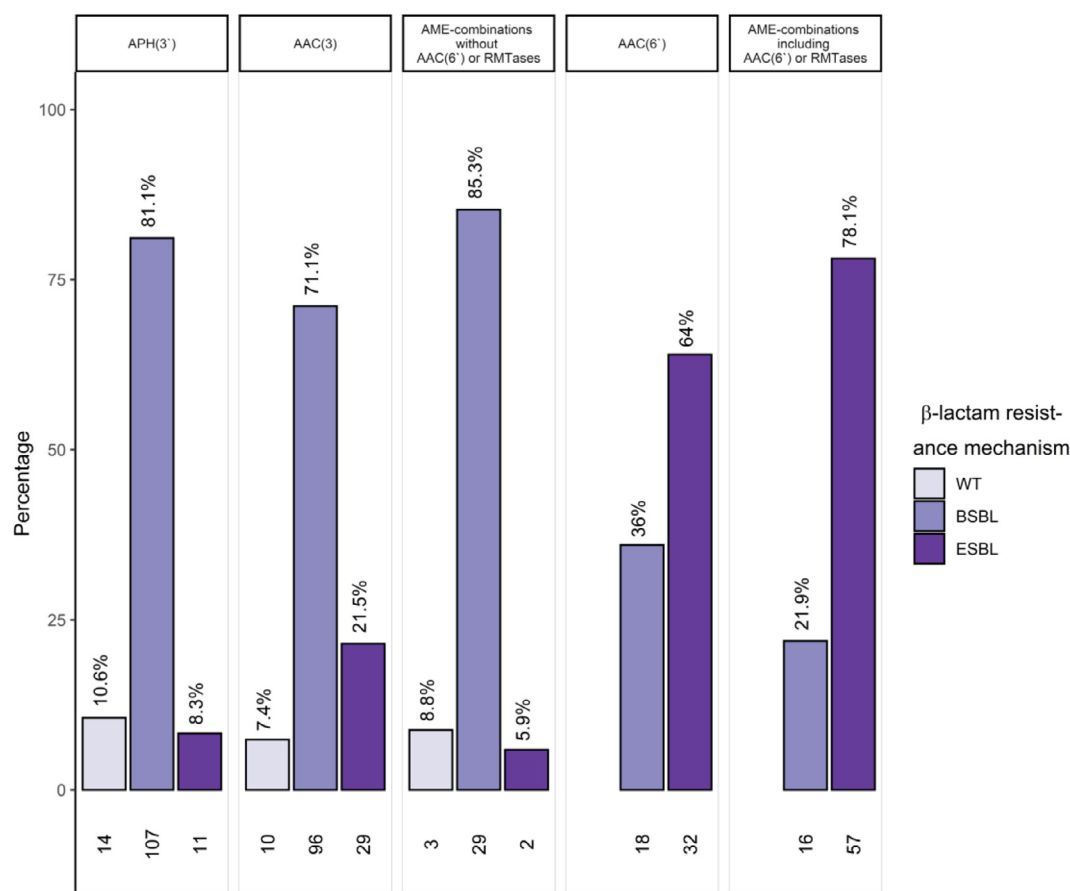
### 3.7. Co-evolution of aminoglycoside and $\beta$ -lactam resistance

The pattern of co-resistance described here is puzzling. In general, the prevalence and co-occurrence of resistance mechanisms most likely reflects the history of antibiotic usage. AMEs



**Table 2**  
Aminoglycoside and  $\beta$ -lactam resistance genes in 439 *E. coli*

[illegible]



**Fig. 2. Co-occurrence of  $\beta$ -lactam and aminoglycoside resistance genes.** BSBL, broad-spectrum  $\beta$ -lactamase; ESBL, extended-spectrum  $\beta$ -lactamase; WT, wild-type. The aminoglycoside resistance mechanism(s) based on WGS data are indicated at the top. The heights of the columns reflect the percentages of  $\beta$ -lactam resistance mechanisms co-occurring within each aminoglycoside resistance group. The numbers at the base of each column indicate the number of strains per category. Eleven AmpC and four AmpC/ESBL producing strains were not included in this figure.

**Table 3**

The most common aminoglycoside-modifying enzymes in this study and their respective spectra of activity [1,8]

ARM	Aminoglycosides modified
APH(3')	KAN
AAC(3)	KAN, GEN, TOB
AAC(6')	KAN, TOB, AMK
RMTases	KAN, GEN, TOB, AMK

ARM: aminoglycoside resistance mechanism, AMK: amikacin, GEN: gentamicin, TOB: tobramycin, KAN: kanamycin.

active against the first marketed aminoglycosides (gentamicin, tobramycin and kanamycin), such as APH(3')-I [34,38] and AAC(3)-II [34,38], were often found in association with BSBLs, which confer resistance towards first-generation  $\beta$ -lactams [6,39]. Indeed, strains resistant to first-generation BSBLs, such as cephalotin, were frequently resistant to gentamicin and tobramycin [40–42]. In contrast, AAC(6')-Ib, which causes decreased susceptibility to amikacin [6,8], an aminoglycoside marketed much later than gentamicin or tobramycin [12], was mostly found in combination with ESBL. A marked increase of amikacin resistance-conferring enzymes, mainly AAC(6'), was seen during the 1980s in regions using amikacin [43]. This coincides with the introduction of ESBLs [39], followed by the emergence and spread of ESBL-producing strains [11,39].

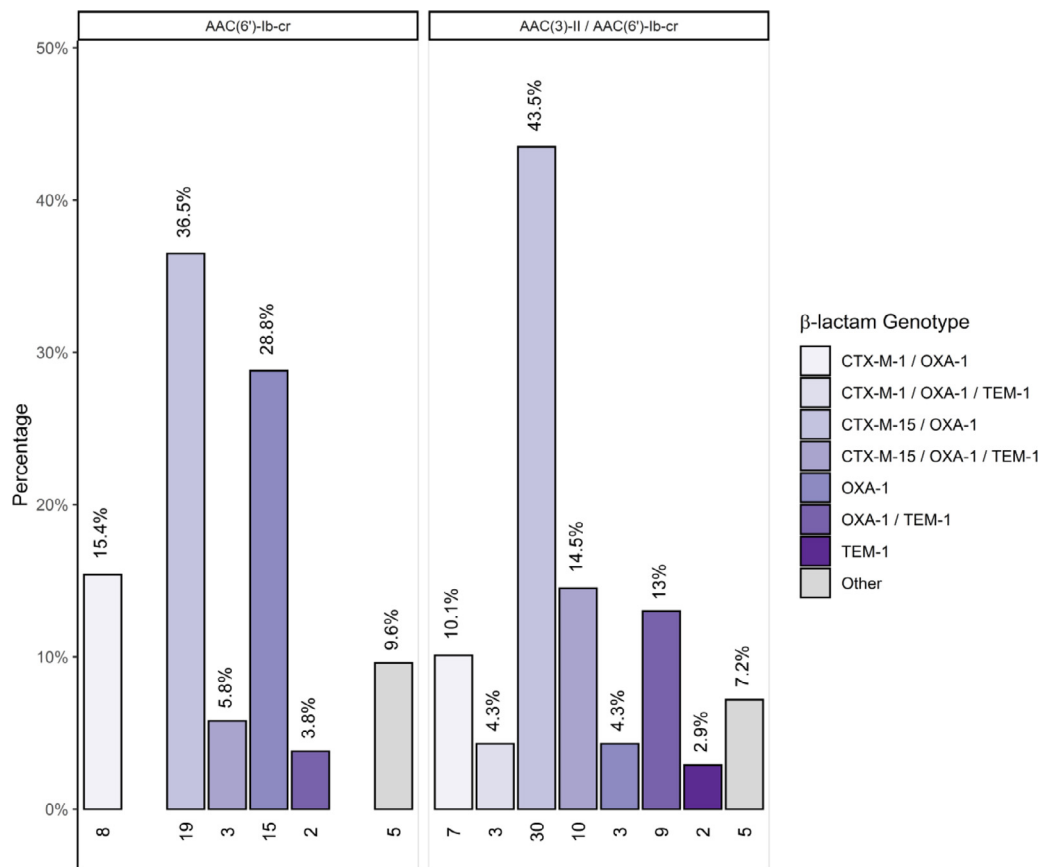
Thus, the evolution of co-occurrence of aminoglycoside and  $\beta$ -lactam resistance mechanisms is ongoing. Beginning in the 1960s with APH(3') and AAC(3) associated with BSBLs, this was followed by AAC(6') associated with ESBLs in the 1980s, and more recently by RMTases associated with carbapenemases [7,44,45]. The latter two mechanisms confer resistance to virtually all  $\beta$ -lactam and aminoglycoside antibiotics currently available in clinical practice, including plazomicin, the most recently developed aminoglycoside antibiotic [46].

### 3.8. Sequence types

To determine whether clonal spread is involved in the co-occurrence of resistance described, the sequence types (STs) of the *E. coli* genomes were analysed. This revealed a wide diversity of 76 STs (Table S3). The most abundant, ST131, was found in 124 strains associated with several  $\beta$ -lactam and aminoglycoside resistance genes. The most frequent genotypes were *aac(3)-IId/bla<sub>TEM-1</sub>* (n=27), *aac(3)-IIa/aac(6')-Ib-cr/bla<sub>CTX-M-15</sub>/bla<sub>OXA-1</sub>* (n=18) and *aac(6')-Ib-cr/bla<sub>CTX-M-15</sub>/bla<sub>OXA-1</sub>* (n=17). STs 69, 141, 10, 1193, 88, 58, 648 and 354 occurred at least 10 times each.

### 3.9. Conclusions

In conclusion, aminoglycoside resistance and the prevalence of AMEs in *E. coli* in the greater Zurich area are comparable to reports from other countries, such as Spain, Poland [4] and



**Fig. 3. Co-occurrence of AAC(6')-Ib-cr and  $\beta$ -lactamases.** 121 strains encoded an AAC(6')-Ib-cr. All AAC(3)-II enzymes were grouped to facilitate an overview.  $\beta$ -lactamases occurring only once were grouped as "Other". The numbers at the base of each column indicate the number of strains per category. Detailed information about each combination is available in Table S4.

Norway. To the best of our knowledge this study is the first to examine the co-occurrence of  $\beta$ -lactamase and AME genes by WGS. Non-susceptibility to aminoglycosides was caused by a remarkable variety of AMEs and was predominantly due to *aph(3')-Ia*, *aac(3)-II* and *aac(6')-Ib-cr*, which are mostly associated with various types of  $\beta$ -lactamases. Non-susceptibility to aminoglycosides was rarely found in  $\beta$ -lactam-susceptible clinical isolates of *E. coli*. The frequent co-occurrence of AMEs/RMTases conferring resistance to all aminoglycosides available needs careful consideration, particularly for ESBL/carbapenemase-producing Enterobacteriaceae.

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### Declarations

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**Competing Interests:** E.C.B. is a co-founder of Juvabis AG, a start-up biotechnology company with an interest in aminoglycoside therapeutics. All other authors: none to declare.

**Ethical Approval:** Not required.

### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ijantimicag.2020.106019](https://doi.org/10.1016/j.ijantimicag.2020.106019).

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